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(54) Title: METHOD OF PROPHYLAXIS OF INFECTION

(57) Abstract: The invention provides a method for prophylaxis of infection of the respiratory tract of a subject by pathogenic airborne bacteria the method comprising administering to the subject by inhalation binding proteins directed against the bacteria. The invention further provides compositions and an inhaler product for use in the treatment method.

METHOD OF PROPHYLAXIS OF INFECTION

This patent relates to the prevention in humans and other mammals of symptoms arising from the presence of air-borne pathogenic bacteria. Such bacteria include *Yersinia* spp, *Mycobacterium* spp, *Brucella* spp, *Bacillus anthracis*, *Chlamydia pneumoniae*, *Coxiella burnetii* and *Legionella pneumophila*.

Background

Kollberg (WO9841235) teaches the use of avian polyclonal antibodies against the bacteria *Pseudomonas aeruginosa* to treat respiratory tract infections caused by this bacteria. The antibody was applied topically to the respiratory tract of children suffering cystic fibrosis. The bacteria was present prior to treatment commencing. The beneficial effect of the antibody was measured by isolating bacteria from the sputum. Prolonged treatment was associated with reduced bacterial counts. *Pseudomonas aeruginosa* does not survive within phagocytic cells. Kollberg did not demonstrate sustained prophylaxis since the antibodies were always applied after the bacteria were present.

Ramisse et al (Journal of Infectious Disease, 1996, May 173(5) 1123-8) teaches the use of polyclonal antibodies against *Steptococcus pneumoniae* to treat respiratory infections in mice caused by this bacteria. The antibody and its fragment were applied either intravenously or intranasally after the mice were infected with the bacteria. *Streptococcus pneumoniae* does not survive within phagocytic cells. Sustained prophylaxis associated with the use of antibodies was not suggested.

Cheng et al (Infectious Immunity 2001, April; 69(4): 2302-8) teaches the use of polyclonal antibodies against group B *Streptococcus* bacteria to increase opsonisation and phagocytosis of this bacteria. Opsonisation is the process where an antibody bound to a bacteria stimulates the activity of phagocytic cells in the locality. This was measured by increased killing of the bacteria by macrophages in cell culture. The increase of phagocytosis is presumably

beneficial *in vivo* however no *in vivo* experiments were reported. *Streptococcus* bacteria do not survive in phagocytic cells. Sustained prophylaxis was not demonstrated.

De Hennezel et al (2000, Antimicrobial Agents and Chemotherapy, 45(1):316 teaches the use of polyclonal antibodies against *Steptococcus pneumoniae* to treat infections in mice caused by this bacteria. The antibody was delivered intranasally and parenterally. The beneficial effect was measured by recording lethality and counting bacteria in lung homogenates. *Steptococcus pneumoniae* does not survive in phagocytic cells. De Hennezel et al did not demonstrate sustained prophylaxis since the antibodies were applied after the bacteria were present.

Collins (US 4,994,269) teaches the use of a monoclonal antibody against *Pseudomonas aeruginosa* to prevent and treat respiratory disease caused by that bacteria. The intranasal use of polyclonal and monoclonal antibodies is described and the beneficial effect was recorded by measuring lethality and counting bacteria in lung homogenates. Pseudomonas aeruginosa does not survive in phagocytic cells. Collins gave the antibody preparations 20 minutes before challenge with the bacteria – this is does not suggest a sustained prophylactic function because it would require dosing to happen very frequently to provide a clinically useful effect.

Eyles et al (Vaccine 1998 Apr; 16(7):698-707) teaches the use of an active intranasal vaccine made of bacterial proteins to stimulate the body's own immune system and to prevent disease caused by the respiratory form of *Yersinia pestis*. *Yersinia pestis* does survive within phagocytic cells. Particular success was seen when cholera toxin B subunit was used as a strong mucosal adjuvant to stimulate an immune response in the mice. The preparation in the above treatment (vaccination) does not comprise antibodies.

Di Genaro et al (1998, Microbiological Immunology; 42(11):781) teaches the use of an active intranasal vaccine made of bacterial proteins to stimulate the body's own defence system and to prevent disease caused by a respiratory

form of *Yersinia enterocolitica* in mice. *Yersinia* does survive within phagocytic cells. The preparation in the above treatment (vaccination) does not comprise antibodies.

For bacteria that survive in phagocytic cells (eg *Yersinia*) the literature does not teach that the topical application of antibodies would result in a useful clinical result. The following concepts also teach away from the topical application of antibodies in managing respiratory disease caused by bacteria that survive in phagocytic cells:

Antibodies which bind with bacteria increase the phagocytosis of those bacteria by immune cells such as macrophages. This process (also known as Fc receptor-mediated phagocytosis) is well described in many medical texts and is known as 'opsonisation' (Huber et al, Journal of Immunology, 2001 Jun 15; 166(12): 7381-8). A consequence of opsonisation is as follows: for bacteria which survive within phagocytes, antibody treatments will be counter-productive because the antibodies will encourage the bacteria to enter a safe haven (the phagocytes).

The known vaccines for protection of mammals against air-borne bacteria that cause respiratory must be given at least weeks before possible exposure to the pathogen of concern to allow the immune system of the body to respond to the vaccine.

Summary

We have made the surprising discovery that sustained prophylaxis against bacterial infection in the mammalian respiratory tract can be achieved by inhalation of binding proteins. By sustained prophylaxis we mean a clinically useful period of prophylaxis of at least one hour and preferably at least 3 hours.

Accordingly we provide a method of prophylaxis of bacterial infection of the respiratory tract of a patient, the method comprising inhalation by the patient of binding proteins.

The invention further provides the use of binding proteins in preparation of a medicament for prophylaxis of bacterial infection of the respiratory tract by administration of the medicament to the respiratory tract.

In a further aspect the invention provides a composition for prophylaxis of bacterial infection of the respiratory tract, the composition comprising binding proteins and a propellant for delivering the composition as an aerosol for inhalation.

In yet a further embodiment the invention provides an inhaler product for prophylaxis of bacterial infection of the respiratory tract comprising a pressurised container enclosing a mixture of a binding protein composition and a propellant, preferably an HFC propellant.

Detailed Description

In one preferment the bacteria are bacteria which survive inside phagocytes and the binding proteins are directed against such bacteria, for example *Yersinia* spp, *Mycobacterium* spp, *Brucella* spp, *Bacillus anthracis, Legionella pneumophila, Cosciella burnetii* and *Chlamydia pneumoniae.*

It is preferred that the binding proteins comprise antibodies or antibody fragments directed against the bacteria. Examples of antibodies and antibody fragments include polyclonal antibodies, monoclonal antibodies, F(ab) fragments, F(ab)2 fragments, antibody tip fragments, chimeric and humanised antibodies and fragments, and recombinant antibodies and fragments.

It is preferred that the antibody preparations are affinity purified. Preferably the binding proteins are formulated in conjunction with other agents which protect the function of the antibodies in hostile environments. Examples of such agents are mammalian colostrum and colostrum extracts which have been described in International Patent Application No. PCT/AU03/00348, the contents of which are incorporated by reference.

In one preferment the antibodies are taken from bovine colostrum or the yolk of a bird egg. Example, antibodies from hyperimmune colostrum (or hyperimmune egg yolk) may be used. It will be understood by those skilled in the art having regard to the above disclosure that suitable binding protein may be prepared as hyperimmune colostrum (or egg yolk) by immunizing a mammal (or bird) with antigen derived from the pathogen.

The method described in this patent gives immediate sustained protection (for at least 1 hour) making it suitable for use immediately before entering a high-risk area.

In one embodiment the invention provides an inhaler composition comprising a mixture of binding protein or proteins and a propellant. The composition may further comprise excipients in addition to the carrier.

The propellant may be a fluorocarbon propellant such as a CFC, HCFC or HFC. Hydrofluorocarbon (HFC) propellants are particularly preferred. Examples of suitable hydrofluorocarbon propellants include HFC-134a and HFC-227.

The carrier for the binding protein is preferably essentially free of water. The carrier may be a finely divided particulate material or a liquid. In many cases the binding protein is moisture sensitive. The composition of the binding protein may be a solution or dispersion in the propellant and the composition may include further solvents such as lower alkanols (eg ethanol) glycerol, lower alkylene glycols or mixture.

The inhaler device of the invention preferably includes a container which maintains the formulation under pressure and is impermeable to the ingress of moisture. The inhaler device may be a single use inhaler or multidose inhaler. A multidose inhaler may be provided with a drug metering valve. The inhaler may contain a moisture absorbing material.

In an alternative embodiment the composition may comprise a finely divided solid composition comprising the binding protein and a liquefied gas. The liquefied gas is preferably an inert gas such as nitrogen or a noble element. An example of a suitable method of formulation in liquid gas is described by Meekka et al in US patent No. 6378518.

In yet another embodiment the binding protein may be in finely divided form comprising particles of for example from 20 nm to 100 microns and preferably 50 nm to 10 microns. The finely divided composition may include anticaking agent.

The inhaler device of the invention may include a compartment containing a powder, a passage for providing an air-stream by inhalation of the user and means for releasing the binding protein powder formulation into the air stream.

The inhaler device may comprise a multiplicity of chambers. In one embodiment for example the device includes one chamber containing a binding protein in dry finely divided form and a second chamber containing a propellant or carrier. The device comprises means for providing mixing of the contents of the first and second chambers. For example, the chambers may be separated by a frangible wall which in operation is breached to provide mixing of the finely divided dry binding protein and the carrier or propellant. Such an arrangement may be particularly useful where the binding protein has a limited shelf life in the carrier or propellant. In this embodiment the inhaler may include means for rupturing the frangible wall. The frangible wall when used may be ruptured by a variety of means such as a piercing plunger or by relative rotation of the different section of an inhaler each provided with one of said chambers.

In a further embodiment of the inhaler of the invention the composition of the binding protein includes a liquid carrier which may be premixed with the binding protein or mixed therewith prior to inhalation and the inhaler comprises a spray nozzle and mechanical means for providing delivery of liquid binding protein mixture to the spray nozzle to form an aerosol.

The dose of binding protein required to provide prophylaxis of infection will depend on the particular bacteria and the risk and of exposure. Typically the dose will be in the range of from 0.1 to 100 milligrams per kilogram of bodyweight of the individual in whom infection is to be prevented. More preferably the dose will be in the range of from 0.8 to 80 mg/kg.

The subject to be treated may be a human or lower animal subject.

The invention will now be described with reference to the following examples. It is to be understood that the examples are provided by way of illustration of the invention and that they are in no way limiting to the scope of the invention.

Example 1

The results of Example 1 are discussed with reference to the attached drawings. In the drawings:

Figure 1(a) is a chart comprising the effect of prophylactic treatment in accordance with the invention with controls; and

Figure 1 (b) examines the statistical significance of the results illustrated in Figure 1(a).

Pernasal administration of specific antibody provides sustained immuno-protection of mice against respiratory challenge with a intracellular bacterial pathogen (*Yersinia enterocolitica)* when given before the bacterial challenge.

In this example *Yersinia enterocolitica* serves as a model for *Yersinia pestis*.

Introduction

The aim of this study was to determine if pernasally administered polyclonal antibodies obtained could provide sustained protection when challenged with an inhaled bacterial pathogen.

The mouse model used in this study involved pernasal inoculation with *Yersinia enterocolitica*, a pathogen that is able to colonise and cause serious pneumonia in the lungs of mice. *Yersinia* bacteria survive within phagocytic cells.

For this study, purified rabbit antibodies (whole IgG or $F(ab')_2$ antibody fragments) directed against *Y. enterocolitica* and a variety of control materials were given pernasally to anaesthetised mice, either 3 hours before or 3 hours after the intranasal challenge with a suspension of 5×10^6 colony-forming units (CFU) of living *Y. enterocolitica* bacteria. The extent of the protection against infection given by the antibodies and their fragments was determined from measurement of bacterial clearance 24 hours after infection.

Experimental procedures

Bacteria

A virulence plasmid – bearing strain of *Y. enterocolitica* O:8 (strain 8081) was cultured in Tryptone Soy broth (Oxoid) for 24 hr at 28°C. Cells were centrifuged and then washed several times following suspension in sterile PBS. Final suspension was prepared to give 5×10^6 CFU in 50 μl of sterile PBS. The actual number of bacteria in the inoculum was determined by plating a sample onto Brain Heart Infusion plates (Oxoid) and counting the CFU after incubation for 24hr at 28 °C.

Mice

Groups of five male Black Ten mice (6-8 week old) were kept in separate cages and were infected within a biosafety cabinet to minimise the airborne spread of *Y. enterocolitica*. Prior to the administration of antibodies and bacteria, mice were anaesthetised briefly with inhaled Penthrane (methoxyflurane). Antibodies and bacteria were administered by placing 50 μl of the appropriate control solution, antibody preparation or bacterial suspension onto the nares of the anaesthetised mouse. The mouse inhaled the drop and the animal was then

allowed to recover. Animals were observed during the recovery period until the righting reflex returned.

Treatment Groups

As detailed in Table 1, test mice received 100 μg of either purified whole rabbit immunoglobulin (IgG) to *Y. enterocolitica* (O:8) or the $F(ab')_2$ fragment of the IgG. Antibodies were given pernasally (in 50 μl solution) to anaesthetised mice in each test group (10 mice per test group). Antibodies were given either 3 hours before or 3 hours after intranasal challenge with a suspension of 5×10^6 CFU of *Y. enterocolitica* serogroup. Control groups of mice (5 mice per control group) received either 50 μl PBS buffer, 100 μg of purified IgG from unimmunised rabbits (Non-specific IgG) or 100 μg of a pool of serum proteins from unimmunised rabbits, after removal of IgG using Protein A chromatography (Non-IgG proteins). An additional group of 5 control mice were given whole IgG, without a bacterial challenge, to assess any histopathological effect of the antibody on the lungs.

Table 1 – Treatment Groups

Group No.	No. of mice	Test material	Dose	Timing of administration of test material –before or after infection
1. Test	10	Whole IgG	100 μg	3 hr before
2. Test	10	Whole IgG	100 μg	3 hr after
3. Test	10	$F(ab')_2$ Fragment	100 μg	3 hr before
4. Test	10	$F(ab')_2$ Fragment	100 μg	3 hr after
5. Control	5	Non specific IgG	100 μg	3 hr before
6. Control	5	Non-IgG protein	100 μg	3 hr before
7. Control	5	Buffer	N/A	3 hr before
8. Control	5	Whole IgG	100 μg	No Infection

Measurement of bacterial clearance from the lung

A comparison of bacterial clearance was made 24 hr after infection to determine the extent of the bacterial clearance induced by the test and control materials.

After mice were killed using inhaled CO_2, the cranial left lobe of the lung was removed aseptically and placed in a preweighed bottle containing 1ml of sterile PBS. The lobe was weighed and then homogenised in the PBS. Serial dilutions were plated on MacConkey agar to estimate counts of the recovered bacteria.

Histopathological studies

The cranial right lobe of the lung was removed from each mouse, together with the heart, kidney, spleen and part of the liver and fixed in 10% formalin. These tissues were dehydrated in alcohol, embedded in paraffin and sections were stained in haematoxylin-eosin. Lung infection was identified by inflammatory cell infiltration into alveoli, bronchiolitis and loss of normal histoarchitecture. In comparison, lung protection was evaluated by the presence of normal histoarchitecture.

Statistics

Data from the estimates of recovered bacteria in the cranial lobe of the lungs were analysed using Student's 2-tailed t test. The estimates of CFU were transformed logarithmically to normalise their distribution and reduce the variance. A *P* value of <0.05 is considered statistically significant.

Results

Bacterial clearance from the lung after passive immunisation

As shown in the attached Figure 1a, bacterial clearance from the lungs of treated mice (groups 1 and 3) was highly significant when compared to control mice ($P<0.001$ vs. group 6 and $P<0.05$ vs. group 5). These results indicate that pernasal administration, 3 hours before infection, of either the whole IgG of rabbit antibodies to *Y. enterocolitica* or the $F(ab')_2$ fragment of the IgG, significantly reduced the numbers of *Y. enterocolitica* recovered from the lung.

This protection surprisingly occurred only when the antibodies were given 3hr before the bacterial challenge but not when given after the challenge.

From Figure 1b which shows the structural significance of the results it is clear that treatments 1 and 3 (in which antibody was administered prior to challenge by the pathogen) are significantly better.

Histopathological studies

There was no evidence of any histopathological effect of the pernasal administration of antibody (without a bacterial challenge) on the lungs of 5 control mice that were given whole IgG.

Example 2

Manufacture of hyperimmune serum against *Yersinia* spp. in rabbits.

A virulence plasmid – bearing strain of *Y. enterocolitica* O:8 (strain 8081) was cultured in Tryptone Soy broth (Oxoid) for 24 hr at 28°C. Bacteria were harvested to yield 5 x 10^6 CFU in 50 μl of sterile PBS. The number of bacteria in the sample was determined by plating a sample onto Brain Heart Infusion plates (Oxoid) and counting the CFU after incubation for 24hr at 28 °C.

The bacteria were spun down, washed in PBS and then heat-killed by boiling. The bacteria were again washed and aliquoted into doses corresponding to the following numbers of whole heat killed bacteria: 1x10^6, 4x10^6 , 8x10^6 , 2x10^7, 4x10^7, 1x10^8. These doses correspond to each vaccine dose in sequence.

1 ml of vaccine was injected into the thigh muscle of 3 rabbits twice a week for 3 weeks. 6 vaccinations were given to each rabbit.

Blood was harvested from the ear vein of the rabbits. After harvesting, the blood was allowed to clot and the hyperimmune serum was removed, aliquoted and frozen at -20ºC.

Example 3

Manufacture of F(Ab2) fragments against *Yersinia* spp.

Immunoglobulin was purified from rabbit antiserum to *Y. enterocolitica* (O:8), using Protein-A chromatography on a Millipore ProSep-A affinity column, with PBS pH 7.4 as the running buffer. Immunoglobulin was eluted with 0.1M Glycine / HCl pH 3.0 buffer, neutralised by addition of 1M Tris solution and then dialysed against PBS pH 7.4. Protein-A chromatography was also used to purify non-specific immunoglobulin from unimmunised rabbit serum for use as "Non-specific IgG" control.

The $F(ab')_2$ fragment of the purified rabbit immunoglobulin to *Y. enterocolitica* (O:8) was prepared by digestion with immobilised pepsin (Pierce Chemical Co.) to remove the crystallisable (Fc) region of the antibody molecule. Prior to digestion, purified immunoglobulin was dialysed against 20mM sodium acetate buffer at pH 4.5 and adjusted to a concentration of 10mg/ml. Immunoglobulin was then incubated with immobilised pepsin at 37°C for 4hrs. Digestion was stopped by centrifugation of the incubation mixture to remove the immobilised pepsin and adjustment of the pH of the supernatant to 7.2. The $F(ab')_2$ fragment was purified from undigested IgG and whole Fc fragments by re-application over a ProSep-A affinity column. Results from the mice receiving the antibody fragments were used to indicate if passive immunity required intact immunoglobulin or only the antigen-binding portion. All antibody preparations and control solutions were adjusted to a protein concentration of 2mg/ml in PBS before administration to mice.

Example 4

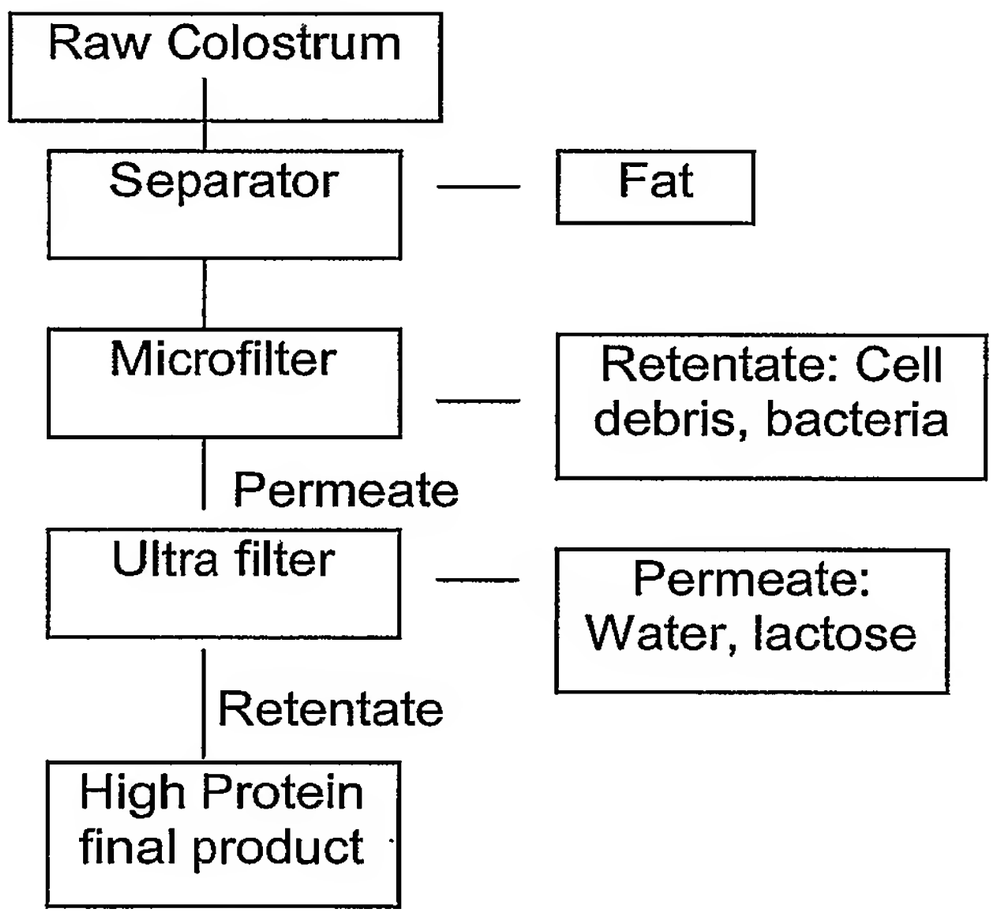
Hyperimmune Colostrum Against Anthrax

Live anthrax vaccine (STERNE strain supplied by Fort Dodge Animal Health a division of Wyeth, located at Overland Park, Kansas City, Kansas, U.S.A.) was used to make hyperimmune bovine colostrum using the following method.

Cows are immunised by a registered veterinarian with a 1m injection of the STERNE strain vaccine as supplied into the muscle tissue on the side of the neck. Up to 5 injections are given at 2 weekly intervals during months 6 to 8.5 of gestation, ceasing 1 month before parturition. Test bleeds are taken from a selection of the immunised cows and assayed to determine the level of specific antibodies. Results of these assays are used to determine if a satisfactory titre has been achieved.

Hyperimmune colostrum was harvested and processed according to the method of Example 2 of International Application No. PCT/AU03/00348.

The following diagram shows the principles used to take colostrum and convert it to a processed form.



The raw colostrum is collected from dairy cows most preferably at the first milking after calving. The colostrum is stored at 4°C on farm and then transported either for longer term storage at –20°C or sent directly to wet manufacturing.

The raw colostrum is warmed to approximately 37°C and then skimmed with a rotary milk separator to remove fat. The resultant liquid may be pasteurised or microfiltered with a 7-10 micron ceramic filter system (to remove bacteria and debris. The liquid is then Ultrafiltered (for example in a Abcor 10m^2 Ultrafiltration plant) to remove a majority of the water, lactose and electrolytes leaving a high protein concentrate. The resultant high protein concentrate is further processed preferably by lyophilization (freeze-drying) or spray-drying.

The above method yield a processed bovine colostrum powder. This product is suitable for inclusion in therapeutic goods.

Example 5

Testing of hyperimmune colostrum for Anthrax binding capacity.

An Enzyme Linked Immuno-sorbent Assay was created using a recombinant protein form of Protective Antigen of *Bacillus anthracis* (PA), sourced from SAPHIRE LABS, an agent of AVANT Therapeutics of Massachusetts, U.S.A., part of the Collier Group.

A test liquor was made by mixing the above bovine colostrum extract powder in water to provide a 2% (by weight) aqueous mixture. This was tested alongside PBS (negative) and a standard mouse monoclonal antibody with a significant affinity for Protective Antigen (*Bacillus anthracis*) using the following assay method. Primary reaction time: 15 minutes.

Assay Method

Method for testing colostrum from cows vaccinated with anthrax vaccine for anti-PA antibodies. (PA refers to protective antigen to *B. anthracis*).

1. One hundred microlitres (100 ul) of a 0.05M Carbonate-bicarbonate buffer containing 1ug of PA /ml was added to the wells of a 96-well microtitre plate, and the plate was incubated overnight at room temperature.

2. The plate was washed (x3) with PBS/0.05% Tween 20.

3. Three hundred microlitres (300 ul) of 3% skim milk in PBS/0.05% Tween 20 were added to each well containing PA to block any sites that did not bind PA. The plate was incubated at room temperature for 1 hr.

4. The plate was washed as above.

5. One hundred microlitres (100 ul) of a 1 in 500 dilution of a 20mg/ml solution of a colostrum sample was added to each well containing PA. The plate was incubated at room temperature for 1 hr.

6. The plate was washed as above.

7. One hundred microlitres (100 ul) of a 1 in 2000 dilution of a goat anti-bovine alkaline phosphatase conjugate was added to each well, and the plate was incubated at room temperature for 1 hr.

8. The plate was washed (x3) with PBS/0.05% Tween 20 and once (x1) with distilled water.

9. One hundred microlitres (100 ul) of a p-nitrophenyl phosphate substrate was added to each well, and the plate incubated at room temperature for 1 hr.

10. Finally, the plate was read at 405nm on an ELISA reader.

The plate also contained a negative (same procedure as the test samples except that 100ul of 3% skim milk in PBS/0.05% Tween 20 was added instead of the colostrums sample) and positive control (same procedure as test samples except 100ul of a 1 in 1000 dilution of a mouse anti-PA monoclonal antibody was added as the sample and a goat anti-mouse alkaline phosphatase conjugate was used instead of the anti-bovine conjugate.

All dilutions were made in 3% skim milk in PBS/0.05% Tween 20.

Results

Assay results are shown in Table 2.

Table 2

Sample	Optical Density
Bovine Colostrum Extract (raised against *Bacillus anthracis*)	3.7
Anti-PA monoclone (positive control)	4.2
Phosphate buffered saline (negative control)	<0.1

The affinity for the bovine colostrum extract for PA is shown by the high optical density which is (a) similar to the positive control and (b) significantly greater than the negative control.

Claims:

1. A method for prophylaxis of infection of the respiratory tract of a subject by pathogenic airborne bacteria the method comprising administering to the subject by inhalation binding proteins directed against the bacteria.

2. A method according to claim 1 wherein the pathogenic bacteria is a bacteria which survives inside phagocytes and the binding proteins are directed against said bacteria which survives inside phagocytes.

3. A method according to claim 2 wherein the binding proteins comprise antibodies or antibody fragments directed against said bacteria which survives inside phagocytes.

4. A method according to claim 3 wherein the binding proteins are selected from the group consisting of polyclonal antibodies, monoclonal antibodies, F(ab) fragments, $F(ab')_2$ fragments, antibody tip fragments, chimeric and humanised antibodies and fragments and recombinant antibodies and fragments.

5. A method according to claim 2 wherein the binding proteins comprise antibodies which have been affinity purified.

6. A method according to claim 2 wherein the binding proteins are administered in conjunction with a protective agent selected from mammalian colostrum and extracts thereof.

7. A method according to claim 2 wherein the binding proteins comprise antibodies taken from colostrum of a mammal or the yolk or an egg.

8. A method according to claim 1 wherein the binding protein is prepared as a hyperimmune protein against a bacteria which survives inside phagocytes wherein the hyperimmune protein is taken from hyperimmune colostrum or hyperimmune egg yolk.

9. A method according to claim 2 wherein the bacteria is selected from the group consisting of *Yersinia spp., Mycobacterium spp., Brucella spp., Coxiella burnetii, Chlamydia pneumoniae, Bacillus anthracis* and *Legionella pneumophila.*

10. A method according to claim 9 wherein the bacteria is selected from *Yersinia spp* and *B. anthracis.*

11. A method according to claim 2 wherein the binding proteins are administered by inhalation as an aerosol.

12. A composition for inhalation as an aerosol for prophylaxis of bacterial infection by a bacteria which survives inside phagocytes the composition comprising binding proteins directed against said bacteria which survives inside phagocytes.

13. A composition according to claim 12 comprising a propellant for delivering the composition as an aerosol for inhalation.

14. A composition according to claim 12 comprising a protective agent for the binding proteins selected from mammalian colostrum and extracts thereof.

15. A composition according to claim 13 wherein the propellant is a hydroflurocarbon propellant.

16. A composition according to claim 12 wherein the binding protein is prepared from hyperimmune colostrum or hyperimmune egg yolk wherein said hyperimmune colostrum or hyperimmune egg yolk was made using an antigen derived from a bacteria which survives inside phagocytes.

17. A composition according to claim 13 comprising a non-aqueous carrier selected from lower alkanols, glycerol, lower alkyleneglycols and mixtures thereof.

18. A composition according to claim 13 in the form of particles of size in the range of from 20 nm to 10 microns.

19. An inhaler product for prophylaxis of bacterial infection of the respiratory tract by a bacteria which survives inside phagocytes the product comprising a chamber containing a composition comprising binding protein directed against said bacteria and a protective agent for the binding protein selected from colostrum and a means for providing an aerosol of the composition.

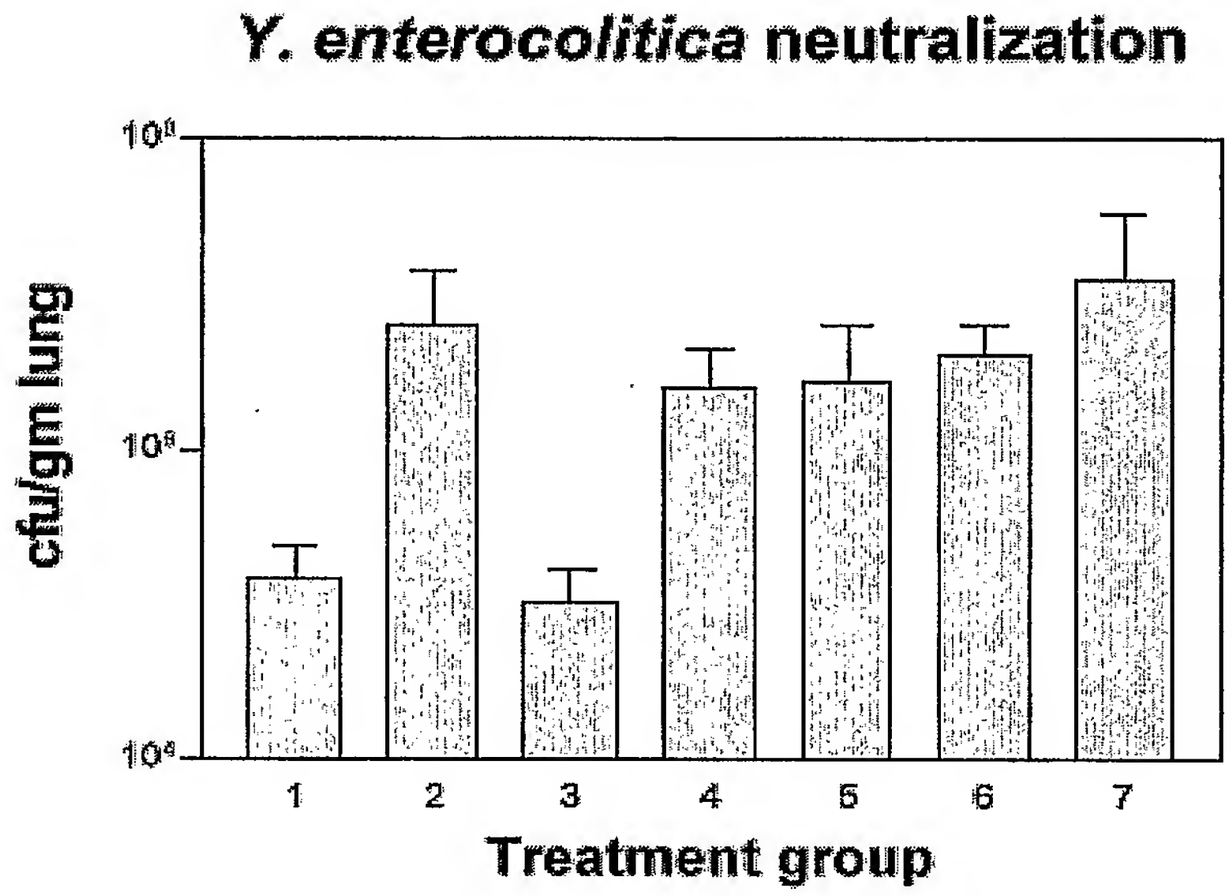
20. An inhaler product according to claim 19 wherein the composition comprising a binding protein is in the form of a powder and the inhaler product comprises a passage for providing an airstream by inhalation of the user and means for releasing the powder composition into the airstream to form an aerosol thereof.

21. An inhaler product according to claim 19 comprising a propellant and a valve for delivering an aerosol of the composition.

22. An inhaler product according to claim 21 comprising a multiplicity of chambers including a first chamber comprising said composition and s second chamber containing a propellant and the inhaler further comprises means for providing mixing of the chambers prior to use.

23. An inhaler product according to claim 22 wherein the composition comprises a liquid carrier or is mixed with a liquid carrier prior to inhalation and the inhaler comprises a spray nozzle and mechanical means for providing delivery of the liquid to the nozzle under pressure.

Figure 1a– Results of Challenge Experiment



Key

Group	Treatment
1	Anti-Y.e. IgG, then Y.e.
2	Y.e., then anti-Y.e. IgG
3	Anti-Y.e. $F(ab)_2$, then Y.e.
4	Y.e., then Anti-Y.e. $F(ab)_2$
5	Non-specific IgG, then Y.e.
6	Non-IgG protein, then Y.e.
7	Buffer, then Y.e.

Stats (Student's t test, 2-tailed)

	1					
2	0.025	2				
3	NS	0.02	3			
4	<0.001	NS	<0.001	4		
5	0.03	NS	0.03	NS	5	
6	<0.001	NS	<0.001	NS	NS	6
7	0.06	NS	0.06	NS	NS	NS

Figure 1b – Statistical Analysis of Results

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU03/00616

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: A61K 39/40, 9/12, A61P 11/00, 31/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MEDLINE, DERWENT; Keywords; Antibody, Ab, Fab, Binding Protein, Inhalation, inhaled, aerosol, nasal, airborne bacteria, yersinia, mycobacterium, brucella, coxiella, bacillus, legionella

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CA 2310365 A (Kings College London) 30 September 2001 abstract, claims, examples (especially example 2)	1-23
X	Ramisse F et al, Passive and Active Immunotherapy for Experimental Pneumococcal Pneumonia by Polyvalent Human Immunoglobulin or $F(ab')_2$ Fragments Administered Intranasally, The Journal of Infectious Diseases, May 1996; 173(5), pages 1123-1128. (Cited in application) abstract	1

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 June 2003	30 JUN 2003
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer **TERRY SUMMERS** Telephone No : (02) 6283 3126

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU03/00616

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4994269 A (Collins et al) 19 February 1991 (Cited in application) abstract	1
P,X	US 2002136695 A (Simon) 26 September 2002 abstract, claims 21-22, 32-33	1
A	WO 0037051 A (Generex Pharmaceuticals Inc) 29 June 2000 abstract	1-23

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU03/00616

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
CA	2310365	NONE					
US	4994269	NONE					
US	2002136695	WO	2002076502	US	2003021778		
WO	200037051	AU	200018518	CA	2354148	EP	1140019
		NZ	512188	US	6312665	US	6375975
		US	6436367	US	6451286	US	2003035831
		AU	200146746	EP	1261320	WO	200166085
		AU	200158112	EP	1296648	WO	200187268
							END OF ANNEX